REVERSAL OF STREPTOMYCIN ACTION

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HALLECK BURKETT WARREN, JR.

B.S., St. Louis University, 1943
M.S., University of Illinois, 1949

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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ACKNOWLEDGMENT

The author wishes to express his sincere thanks to Professor G. I. Wallace for suggesting this problem and for his guidance and helpful suggestions throughout this work. A note of appreciation is extended to several graduate students for their assistance.

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Introduction

Studies on the action of the antibiotic streptomycin have led investigators to pursue diversified means of attack. The inactivation of streptomycin by cysteine and 2-aminoethanethiol was reported (1) soon after the discovery of the antibiotic and later brain tissue and plant extracts were found to be antagonistic to streptomycin (3). This work is concerned with the interference of streptomycin action by various materials commonly used in bacterial media and the response of various bacteria to these materials.

Experimental And Results

Brain infusion was added to a basal medium in amounts to give a final concentration of 0.1 mg, 0.5 mg, 1 mg, and 2 mg per ml. The ability of bacteria from a large number of species representing diverse groups to grow in the presence of brain infusion and inhibitory concentrations of streptomycin was determined by the presence of turbidity. An alcoholic extract of brain was treated in a like manner. Results showed that the inhibition of streptomycin action by brain infusion and an alcoholic extract of brain was general among bacteria.

Difco brain heart infusion is known to exert an antistreptomycin effect (3). Studies were carried out to see if the antistreptomycin effect could be enhanced by the culturing of organisms in this medium. Fourteen organisms representing various groups were cultured in individual portions of Difco brain heart infusion broth. Following periods of abundant growth, the organisms were removed by centrifugation and the supernatant sterilized by passing through an ultra-fine scintered glass filter. The assay for a streptomycin antagonist consisted of adding 0.5 ml of streptomycin solution of desired concentration to 4.5 ml of the broth from the organism under test, and inoculating the mixture with three drops of a 24-hour

broth culture of *Micrococcus pyogenes* var. *aureus* (FDA 209). This procedure was used for streptomycin concentrations of 10, 25, 50, and 75 units per ml upon the filtrate from each of the fourteen organisms. Fresh Difco brain heart infusion broth of corresponding streptomycin concentrations served as controls. Results indicate that during growth bacteria in general impart some factor or factors to the medium capable of reducing the effectiveness of streptomycin beyond that observed with Difco brain heart infusion.

A study was made on the ability of organisms to produce an antistreptomycin factor in Bacto Wolf's casamino acid medium, a medium exerting little interference with the activity of streptomycin. Both the cells and the filtrate of eleven organisms were assayed for the factor. Dried cells and dried filtrate were added to the casamino acid medium to give final concentrations of 1.25 mg and 2.50 mg per ml, respectively. A mixture of 4.5 ml of the casamino acid medium containing either dried cells or dried filtrate and 0.5 ml of streptomycin solution was inoculated with one drop of a 24-hour broth culture of *Micrococcus pyogenes* var. *aureus* (FDA 209). The streptomycin concentrations used were the same as above. The controls were identical to the assay tubes with the omission of dried cells or dried filtrate. Results showed that the antistreptomycin activity in the dried cells or dried filtrate of an organism corresponds to the organism's resistance to streptomycin. With different organisms the antistreptomycin activity may be in the cells, the filtrate or both.

No protection against streptomycin inhibition was afforded the bacterial cells following exposure to Difco brain heart infusion broth for periods up to one hour. This indicates that the antistreptomycin factor is not in competition with streptomycin for adsorption to the cell surface, but that a period of adaptation to the interfering substance in brain tissue is necessary to establish an alternate pathway of metabolism offering resistance to streptomycin.

It was found possible to expose bacteria to streptomycin for varying lengths of time up to 72 hours and obtain a revival

of growth by transferring the inhibited organisms to a medium containing Difco brain heart infusion. Plain nutrient broth failed to produce a revival of growth. The total revival value of Difco brain heart infusion could be only partially accounted for in any of its individual constituents or in any of the different combinations of the individual constituents, thus eliminating the salt content as the sole cause. Phosphate was conspicuous by its presence in various combinations showing slight revival effect. If the statement that streptomycin prevents the formation of adaptive enzymes, which are themselves relatively insensitive to streptomycin (2), may be taken as correct a plausible explanation could be offered for the inability of organisms to revive after 72 hours exposure to streptomycin. Seventy two hours may also be the minimum time limit for streptomycin to inhibit the formation of adaptive enzymes which utilize the antagonistic substance in brain heart infusion.

The study of the revival effect in Difco brain heart infusion was extended to 10 different organisms ranging from streptomycin-sensitive to streptomycin-resistant species. A correlation was found to exist between the amount of the revival effect and the amount of streptomycin necessary to bring about inhibition.

The revival effect of thirteen peptones along with lipositol and sodium thioglycolate upon *Micrococcus pyogenes* var. *aureus* (FDA 209) was investigated. Results showed lipositol to be low in activity while variation occurred among the different peptones.

A patented process for drying and defatting tissue was used to study the protein and fat fractions from beef brain. The revival effect of the protein and fat fractions was compared with that of brain infusion and Difco brain heart infusion. Two test organisms were used, one moderately sensitive to streptomycin and the other highly sensitive to streptomycin. The revival activity in decreasing order was Difco brain heart infusion, brain infusion, and defatted brain

while brain fat showed no effect. Oddly enough, brain infusion produced the greatest antistreptomycin activity but was second to Difco brain heart infusion with respect to the revival of growth.

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VITA

Halleck Burkett Warren, Jr. was born in St. Louis, Missouri on September 3, 1922. After graduating from Breese High School, Breese, Illinois in June, 1940, he entered St. Louis University and received the degree of Bachelor of Science from that institution in September, 1943. He enlisted in the United States Naval Reserve in December, 1942 and was called to active duty September, 1943. He served 34 months on active duty, 23 months being in the Pacific Theater, and was released to inactive duty July, 1946 with the rank of Lieutenant junior grade. The Master of Science degree in Bacteriology was conferred on the author in February, 1949 by the University of Illinois. He held a half-time graduate assistantship in the Department of Dairy Science from July, 1949 until September, 1950, assisting in courses of beginning and advanced Dairy Bacteriology. He is a member of Sigma Xi, Phi Sigma, the Society of Illinois Bacteriologists, and the Society of American Bacteriologists.

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